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QUANTIFICATION OF BACTERIA IN ISOLATED PILOSEBACEOUS FOLLICLES IN NORMAL SKIN

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A technique for quantitating bacteria in isolated pilosebaceous follicles is described. This involves microdissection of the follicles from biopsies of skin, using the method of chemical pretreatment of skin to facilitate the separation of the epidermis and epidermal appendages from the dermis. The aerobic cocci and anaerobic diphtheroids in pilosebaceous follicles in 66 biopsies of scalp and 48 biopsies of skin of the upper back were quantitated using this technique.

On the back, aerobic staphylococci were very sparse in normal follicles, indicating that their primary habitat on the skin must be on the skin surface rather than within follicles. Of 138 isolated follicles from skin of the upper back, 94 contained no aerobic cocci.

Anaerobic organisms were present in high numbers within normal follicles. The geometric mean density of anaerobes in 138 isolated follicles from skin of the upper back was 3.8×10^4 diphtheroids per follicle. Eighty-eight follicles contained more than 10^4 anaerobic diphtheroids.

Using data from scalp biopsies we found that there was a correlation between the weight of sebaceous glands and the density of anaerobes within the follicles attached to these glands (coefficient of correlation = 0.6).

One limitation in analyzing and quantitating the bacteria in human skin has been the lack of a technique for studying the contents of isolated pilosebaceous follicles. On the basis of previous histologic studies it has been assumed that anaerobic diphtheroids proliferate predominantly in the deeper parts of the follicles and that the aerobic organisms reside in the upper levels of the follicles [2]. With one exception, no actual quantitative cultures of normal follicular contents have been described in the literature. The exception was the study by Whiteside and Voss [3] in which the bacterial content of the wormy extrusions produced by pressure extraction of the large follicles in the skin of the nose was analyzed. Whether the contents of these large follicles are representative of smaller follicles in other areas of skin is questionable. Most studies that have attempted to quantitate skin bacteria in normal and diseased conditions have used the buffer scrub method [4] or a modification of it. Such studies have assumed that some proportionality exists between the follicular and the surface flora and thus the latter can be used as representative for the former. The validity of this assumption has never been tested. In fact, when the buffer scrub method was proposed as the most suitable method for sampling surface flora, it was also stated that this method "does not sample the organisms which may be deep within the follicles. . ." [4].

Recently, Holland and Roberts [5] described a method involving cyanoacrylate gel impressions for "sampling microorganisms from the pilo-sebaceous ducts." A drop of cyanoacrylate gel was put on the skin surface within the confines of a teflon ring. A circular disc of 12 mm diameter, which fitted into the ring, was then pressed on the gel for 20 sec, after which the gel with the adhering epidermal cells and follicular contents was removed, homogenized, and quantitatively cultured. This method is not entirely suitable for sampling the contents of isolated sebaceous follicles. For one thing, it would be difficult to determine what proportion of the follicular content was actually removed, since the follicular plug could easily break in the process of being extracted. Also, the surface flora attached to the gel is included in the sample, thus total flora rather than only follicular flora is cultured.

In 1966, Kellum [6] described a procedure for isolating sebaceous glands by microdissection from skin after pretreating skin biopsies in 1 M $CaCl_2$ solution for 2 hr. Chemical separation of the epidermis from the dermis is dependent on the swelling effect on collagen by the calcium and chloride ions. This allows the epidermis with the attached appendages to be gently peeled from the

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dermis. Under the dissecting microscope the various parts of the pilosebaceous apparatus can be dissected and analyzed separately.

In this paper we present a method for quantitating bacterial contents of isolated pilosebaceous follicles from normal skin using Kellum's method of chemical pretreatment of skin as a tool for isolating the follicles. This procedure has been used to quantitatively analyze the bacterial flora within vellus hair follicles and sebaceous follicles of skin from the upper back of normal volunteers, and within hair follicles from the scalp of patients undergoing hair transplant operations. The bacterial flora of the skin surface in areas adjacent to the biopsied sites has also been quantitatively analyzed, as has the relationship between the weight of the sebaceous glands and the density of the anaerobic organisms within the follicles attached to these glands.

The questions our study was designed to resolve were: (1) What numbers of bacteria are present in the pilosebaceous follicles of normal human skin? (2) Is there a correlation between the surface flora sampled by the buffer scrub method, and the deeper follicular flora? (3) Is there a relationship between the size of the sebaceous glands and the density of the bacterial flora within the pilosebaceous follicles attached to these glands?

MATERIALS AND METHODS

Analysis of the effect of 1 M $CaCl_2$ solution on cutaneous bacteria. Evidence that 1 M $CaCl_2$ solution does not inhibit the survival of cutaneous bacteria is essential for the validity of results which are dependent on the use of 1 M $CaCl_2$. To establish this, random isolates of *Propionibacterium acnes*, *P. granulosum*, and *Staphylococcus epidermidis* were grown on brain heart infusion (BHI) agar supplemented with 1% dextrose. The organisms were harvested, washed in 0.85% NaCl solution, and resuspended in 5 ml of saline. Half a ml of each suspension was aliquoted into two test tubes. To one of these, 7 ml of 1 M $CaCl_2$ solution was added, to the other 7 ml of 0.85% NaCl solution. The suspensions were mixed and maintained in the refrigerator at 4°C for 4 hr. Tenfold dilutions in saline solution were made from each of the suspensions and were plated on BHI agar plus 1% dextrose for colony counts using the calibrated drop plate method [7]. Cultures were incubated for 48 hr for the aerobes and 72 hr for the anaerobes.

Biopsies. Sixty-six 3.5-mm punch biopsies were obtained under local lidocaine anesthesia from the bald frontal areas of the scalp of 11 patients undergoing hair transplant operations. All patients were males, ranging in age from 22 to 54 years, with the average age of 38.6 years. Forty-eight 3-mm punch biopsies were obtained from skin of the upper back of 27 healthy volunteers. Eleven were male, and 16 were female. The ages ranged from 19 to 35 years, with average age of 24.9 years. None of the subjects sampled had been on antibiotic treatment during the previous 3-month period.

Biopsies were immediately rinsed in cold sterile saline, placed into individual 1-ml beakers containing sterile 1 M $CaCl_2$ solution, and maintained in 1 M $CaCl_2$ solution at 4°C for 2 hr. After this, each biopsy was immediately dissected. Dumont number 3 and 5 microforceps, and a Circon number 185 ultra microknife (1.5-mm edge) with a 3-cm 30° shaft and a straight handle were used for dissection. Sterile slides, solutions, and dissecting instruments were used throughout.

Isolation of the follicles. The epidermal disc with attached pilosebaceous follicles was peeled from the dermis and placed into a 50-μl drop of water on a glass slide. A single follicle was cut off, the attached sebaceous glands were severed from the follicular duct and removed to a preweighed slip (1 cm^2) of wax paper. The isolated duct was transferred to a microtissue grinder containing 1 ml of 0.1% Triton X-100 in phosphate buffer. The epidermal disc with the remaining follicles attached was transferred to a fresh 50-μl drop of water and the next follicle dissected in a manner similar to that just described.

Thus, each follicle was cut off in a fresh drop of water. Five microliters of the water remaining on the slide were removed with an automatic micropipette for dilution and quantitative culture to determine the percentage of organisms released into the dissecting solution during sectioning of the follicles. The epidermal disc remaining after the follicles had been removed was processed in a manner identical to the individual follicles.

Processing of follicles. Depending on the experiment, either of following procedures was followed: (1) All of the follicles from a single biopsy were pooled together into one homogenization tube and processed together. The sebaceous glands attached to these follicles were pooled together on a 1-cm^2 slip of tared weighing paper, dried overnight in desiccator jars, and weighed on a Cahn balance Model G-2 the following day. (2) Isolated follicles were placed into individual homogenization tubes and were processed separately. In both procedures, the number of follicles in each biopsy was recorded.

Follicles were homogenized for 1 min in Elvehjem microtissue grinders (1-ml capacity) or in Tenbroeck microtissue grinders (2-ml capacity) using 1 ml of 0.1% Triton X-100 in 0.75 M phosphate buffer as the homogenizing solution.

Surface flora. At the same time as the biopsies were obtained, the surface flora from an area of skin immediately adjacent to the biopsied site was sampled using the buffer scrub technique [4]. Two consecutive 1-ml washes of 0.1% Triton X-100 phosphate buffer were made by pipetting the buffer solution into glass cylinders (3.8 cm^2 area) and rubbing the area within the cylinders with rubber policemen for 1 min.

Bacterial cultures. Each sample to be cultured was diluted 5 times in 10-fold dilutions with BHI broth. Five to 6 drops of each dilution were spotted on duplicate plates of BHI agar supplemented with 1% dextrose using calibrated micropipettes according to the method of Reed and Reed [7].

One set of plates was incubated aerobically at 37°C for 48 hr, the other set anaerobically at 37°C under 90% CO_2 and 10% nitrogen, for 5 days. Resulting colonies were classified according to colonial morphology and Gram stain as aerobic cocci or anaerobic diphtheroids without further classification of the cocci into subgroups according to the Baird-Parker scheme or into species of propionibacteria as *P. acnes* or *P. granulosum*. Species other than aerobic cocci and anaerobic diphtheroids were not commonly isolated. In instances where they occurred, the counts were noted, but they were not used for analysis of the results.

Results were calculated as the number of organisms per cm^2, the number of organisms per biopsy, or the number of organisms per follicle.

TABLE I. *Effect of 1 M calcium chloride treatment of cutaneous bacteria compared to treatment with physiologic saline*

Strain		Bacterial counts of duplicate samples after $CaCl_2$ treatment		Bacterial counts of duplicate samples after NaCl treatment	
S. epidermidis	1	5.0×10^8	4.0×10^8	3.2×10^8	2.4×10^8
	2	3.4×10^8	5.0×10^8	4.8×10^8	4.6×10^8
	3	2.6×10^7	3.2×10^7	4.2×10^7	2.8×10^7
	4	2.0×10^8	3.2×10^8	2.7×10^8	2.8×10^8
	5	1.2×10^8	1.2×10^8	2.2×10^8	1.3×10^8
P. acnes	4	5.0×10^7	5.4×10^7	4.2×10^7	5.6×10^7
	6	6.6×10^7	6.6×10^7	6.0×10^7	8.0×10^7
	18	2.4×10^7	3.2×10^7	2.8×10^7	3.0×10^7
	142	3.2×10^7	2.0×10^7	3.4×10^7	2.6×10^7
	154	5.2×10^7	4.6×10^7	4.8×10^7	5.0×10^7
P. granulosum	D21	4.2×10^4	2.5×10^4	7.3×10^3	1.1×10^4
	D22	1.2×10^6	1.7×10^6	4.8×10^6	4.6×10^6
	D34	3.5×10^6	3.4×10^6	4.3×10^6	5.4×10^6
	C54	1.7×10^4	1.8×10^4	1.8×10^4	1.6×10^4
	V61	8.0×10^5	1.1×10^6	6.0×10^5	6.6×10^5

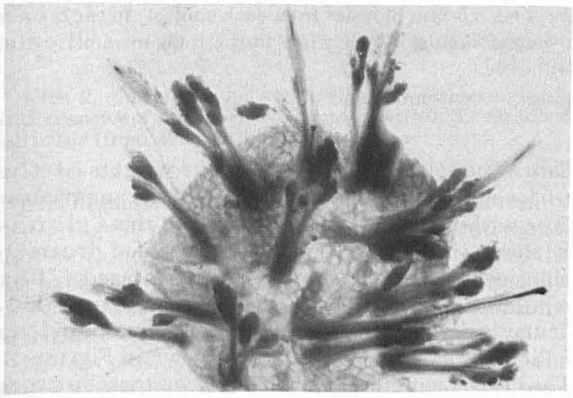

FIG. 1. Epidermal disc from scalp skin with hair follicles attached.

RESULTS

Effect of 1 M $CaCl_2$ on cutaneous bacteria. Treatment of suspensions of randomly selected cutaneous bacteria with 1 M $CaCl_2$ solution had the same effect on the survival of the organisms as treatment with physiologic saline. Results of colony counts for the 15 strains tested are shown in Table I. Differences in counts between 1 M $CaCl_2$-treated and saline-treated organisms were not significant (according to the F test). Thus, pretreatment of biopsies in 1 M $CaCl_2$ solution to facilitate microdissection of pilosebaceous ducts should not affect the count of the intrafollicular flora.

Isolation of the follicles. Calcium chloride is an effective agent in facilitating the separation of epidermis and epidermal appendages from the dermis. With care the entire epidermal sheet with the attached hair follicles and sebaceous apparatus can be peeled intact from the dermis (Fig. 1).

Fifty-microliter drops of sterile water were used for immersion of the epidermal discs and attached follicles during the dissection procedures. As described under *Materials and Methods*, after one follicle was severed, the disc with the remaining follicles was transferred to a fresh drop. Five-microliter samples of the water remaining on the slides were cultured to estimate the percentage of organisms lost from each follicle during dissection. It was found that there was no correlation between the number of organisms in the dissecting fluid and the number of organisms cultured from the corresponding follicles. There was, however, good correlation between the number of organisms cultured from the epidermal disc, and the organisms cultured from the dissecting fluid. On the average, 10% of the organisms on the epidermal disc were lost into the dissecting fluid.

Scalp. The geometric and arithmetic mean densities of the aerobic and anaerobic bacterial flora within the pilosebaceous follicles of the scalp are shown in Table II. For each subject the figures represent averages of results obtained from 6 individual biopsies, each of which contained, on the average, 19 sebaceous follicles (with a range of 15 to 23 follicles per biopsy). The follicles from each biopsy were pooled and processed together.

Aerobic. The aerobic intrafollicular flora of the scalp of 11 subjects tested had a geometric mean (GM) density of 9.2×10^1 cocci per 3.5 mm biopsy. This value was less than the GM density of the surface flora which was 5.7×10^2 cocci per 0.1 cm^2. Surface flora was expressed per 0.1 cm^2 because this is the area of the epidermal disc of a 3.5-mm biopsy of skin. There was no significant correlation between the aerobic intrafollicular and the aerobic surface flora of the scalp (coefficient of correlation was 0.42).

Anaerobic. The GM density of the anaerobic intrafollicular flora of the scalp was 5.3×10^5 organisms per 3.5-mm biopsy and the GM density

TABLE II. *Comparison of the densities of the surface[a] flora and the intrafollicular[b] flora of the scalp in 11 subjects*

Subject	Aerobic count			Anaerobic count		
	Surface (/0.1 cm²)	Intrafollicular		Surface (/0.1 cm²)	Intrafollicular	
		GM	AM		GM	AM
1	2.1×10^1	2.8×10^2	1.4×10^3	4.2×10^3	contam.	contam.
2	1.6×10^3	7.5×10^2	7.5×10^3	4.2×10^4	2.0×10^6	2.1×10^6
3	7.5×10^3	7.4×10^1	1.1×10^2	1.0×10^4	contam.	contam.
4	2.1×10^4	5.7×10^2	4.8×10^3	2.3×10^4	1.6×10^6	1.7×10^6
5	2.1×10^3	1.8×10^2	1.0×10^3	8.2×10^4	1.7×10^7	1.9×10^7
6	2.1×10^4	8.7×10^1	4.9×10^2	3.1×10^5	1.7×10^4	6.6×10^4
7	7.5×10^2	3.0×10^1	3.3×10^1	9.2×10^4	5.5×10^6	1.0×10^7
8	1.4×10^1	1.5×10^1	2.3×10^1	3.3×10^4	3.7×10^4	6.1×10^4
9	1.1×10^4	2.2×10^2	2.5×10^2	2.1×10^5	2.3×10^5	2.4×10^5
10	3.1	2.4×10^1	2.6×10^1	1.0×10^4	4.6×10^4	2.1×10^5
11	2.1×10^1	1.1×10^1	3.3×10^1	1.5×10^5	1.5×10^6	2.3×10^6
GM	5.7×10^2	9.2×10^1	1.4×10^3	4.3×10^4	5.3×10^5	4.0×10^6
AM	5.9×10^3			8.8×10^4		

[a] Surface flora was calculated per 0.1 cm² since this is the surface area of the epidermal disc from a 3.5-mm biopsy.

[b] Intrafollicular flora was calculated from the average values of six 3.5-mm biopsies from each subject. In each case the follicles from a single biopsy were pooled together. The "intrafollicular" thus refers to the total intrafollicular bacteria in a 3.5-mm biopsy, not the bacteria within a single follicle.

Key: GM = geometric mean; AM = arithmetic mean; contam. = contaminated

TABLE III. *Weight of sebaceous glands compared to the geometric mean density of the anaerobic flora within the corresponding follicles (in scalp)*

Subject	Ave. total wt. of glands[a] (μg)	GM density of anaerobic intrafollicular flora[b]
2	411	2.0×10^6
4	425	1.6×10^6
5	650	1.7×10^7
6	393	1.7×10^4
7	426	5.5×10^6
8	220	3.7×10^4
9	355	2.3×10^4
10	471	4.6×10^4
11	510	1.5×10^6

[a] Average weight of pooled sebaceous glands dissected from each of 6 biopsies in each subject.

[b] Geometric mean densities of the anaerobic flora within the pilosebaceous ducts harvested from each of 6 biopsies in each subject.

of the anaerobic surface flora was 4.3×10^4 anaerobes per 0.1 cm². Using the GM values, the intrafollicular anaerobic flora was 12.8 times the surface values. Using the arithmetic mean values, the intrafollicular anaerobic bacterial densities were 46 times the surface values.

There was no correlation between the density of the intrafollicular flora of the scalp and the density of the surface flora in adjacent areas of scalp (coefficient of correlation was −0.16).

Sebaceous gland weight and follicular bacterial flora. The relationship between the weights of the sebaceous glands and the densities of the anaerobic flora within the follicles connected to these glands is shown in Table III. Values obtained from 6 biopsies from each subject were averaged. The relationship of gland weights compared to intrafollicular microbial densities for the corresponding follicles in each of 52 biopsies is shown in Figure 2. The coefficient of correlation between these factors was 0.6. Thus, there is a trend to heavier microbial infestation of the larger pilosebaceous follicles, or at least the follicles supplied by the larger sebaceous glands. Dry weight of the glands in this case was used as the indicator of gland size.

Back. Comparison of the densities of the aerobic and anaerobic flora of the skin surface and in follicles of the upper back are shown in Table IV. Results in each subject are expressed as total number of bacteria cultured from the individual follicles isolated from each of two biopsies, compared to the number of surface bacteria cultured from an area of skin comparable to the surface area of the biopsies, i.e., 0.14 cm² for two 3-mm biopsies. In the first 9 subjects the intrafollicular flora was quantitated by pooling individual follicles from two biopsies and processing these together. In the remaining 18 subjects each follicle was processed individually. Altogether 48 biopsies with a total of 138 isolated follicles were analyzed. The GM density of the aerobic organisms was 4 cocci and of the anaerobic organisms 3.8×10^4 propionibacteria per individual follicle. Of the 138 follicles cultured, 94 contained no aerobic organisms, and 88 contained more than 10^4 anaerobic

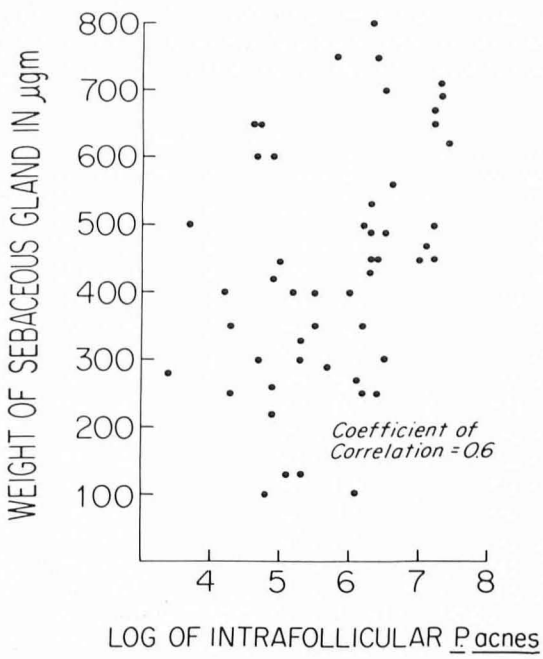

FIG. 2. Correlation between weight of sebaceous glands and density of the anaerobic bacteria within the attached follicles (from 52 scalp biopsies from 9 subjects).

diphtheroids. Figure 3 is a graphic illustration of the range of anaerobic and aerobic bacterial densities within individual follicles. Table V shows the variation of bacterial counts within different follicles obtained from two biopsies from the same subject.

In processing the biopsies from the back, the discs of epidermis left after the follicles had been removed were homogenized and cultured. The GM densities of the bacterial flora of the epidermal discs was 1.0×10^1 for the aerobic and 9.7×10^3 for the anaerobic organisms per 0.07 cm^2 (i.e., surface area of one 3-mm biopsy). These values are very close to the values obtained by the surface scrub method on the back. Using this method the GM densities were 1.8×10^1 for the aerobic and 7.0×10^3 for the anaerobic organisms per 0.07 cm^2.

Good correlation was found between the density of the anaerobic surface flora and the anaerobic intrafollicular flora of the back (coefficient of correlation was 0.69). This suggests that anaerobic bacteria on the skin surface are brought to the skin surface from within the follicles by the constant flow of sebum. There was no correlation between the density of the aerobic surface flora and the intrafollicular aerobic flora.

DISCUSSION

The major disadvantage of the technique we have described is that it is dependent on obtaining biopsies of skin for sampling. Thus, it is not practical for routine bacteriologic evaluations of skin flora. Even in subjects willing to undergo biopsies, one is limited to analyzing a minute area of skin. However, this method is useful for selective studies. It is the first method described which permits absolute quantitation of the bacteria within isolated sebaceous follicles.

It was of utmost importance to establish that 1 M $CaCl_2$ per se has no adverse effect on the survival of the cutaneous bacteria within their intrafollicular habitat in vitro. Experiments with randomly chosen strains of the species representing the typical cutaneous bacteria involved maintenance of these organisms in 1 M $CaCl_2$ solution for 4 hr, and demonstrated that survival was the same as with duplicate suspensions in physiologic saline. During the pretreatment of skin biopsies with 1 M $CaCl_2$, incubation is carried out for 2 hr and there is less direct exposure of the organisms to the $CaCl_2$ solution. Thus, treatment of biopsies with $CaCl_2$ should not modify the bacterial counts within the follicles.

We are not yet convinced of the effect of 1 M $CaCl_2$ solution on lipophilic yeasts. In in vitro studies on the effect of 1 M $CaCl_2$ solution on 5 different stock strains of *Pityrosporum ovale* and *P. orbiculare*, the colony counts were slightly reduced after incubation with $CaCl_2$ solution. However, this may have been an artifact since clumping and agglutination of the organisms occurred in $CaCl_2$ suspensions.

In our initial studies we attempted to culture follicular homogenate suspensions on Mycosel agar supplemented with 1% Tween 80. This is the medium routinely used for maintenance of our stock strains of Pityrosporum species. Growth from follicular homogenates was invariably negative, even though we could confirm the presence of the lipophilic yeasts within the follicles histologically. This confirms the experience of other investigators who have attempted and failed to obtain quantitative cultures of lipophilic yeasts on primary isolation [8].

In the present study of normal follicles we found that *P. acnes* may be quite dense, but that staphylococci are not found with any regularity within isolated follicles. In the only other study in which the contents of normal follicles were analyzed, although these were the slightly unusual large follicles from skin on the nose, very few staphylococci were found in the follicular contents [3].

This is in contrast to the bacterial flora of lesions in acne vulgaris. Numerous studies over the past 15 years [9–12] are in general agreement that all types of acne lesions (open and closed comedones, pustules, and cysts) contain either a mixed population of *P. acnes* and *S. epidermidis*, or pure cultures of either. The most frequent finding in most lesions is a combination of staphylococci and propionibacteria, and 100% of comedones are thought by some to contain both these species [13]. Furthermore, the aerobic cocci in acne lesions are predominantly *S. epidermidis* Baird-Parker biotype 1, even

TABLE IV. *Comparison of the surface flora and intrafollicular flora in skin of the upper back of 27 normal subjects*

Surface count is expressed as the number of bacteria per 0.14 cm^2 (the epidermal surface area of two 3-mm biopsies). The intrafollicular count was calculated by totaling the bacterial counts obtained from all the individual follicles dissected from each of two 3-mm biopsies.

Subject	No. of follicles	Aerobic count		Anaerobic count	
		Surface	Intrafollicular	Surface	Intrafollicular
1	5	2.9×10^1	0	1.4×10^5	5.6×10^5
2	8	4.8	0	2.9×10^2	1.2×10^3
3	12	1.4×10^1	0	1.4×10^5	8.0×10^5
4	7	1.4×10^1	3.2×10^2	7.3×10^4	1.2×10^6
5	7	1.4×10^1	0	1.4×10^4	6.0×10^5
6	6	1.4×10^1	0	1.1×10^4	4.0×10^5
7	5	7.2×10^1	1.3×10^2	5.8×10^2	6.0×10^5
8	7	9.6	1.3×10^2	8.8×10^4	3.6×10^5
9	4	1.4×10^1	2.0×10^2	8.8×10^2	1.3×10^2
10	4	1.4×10^1	6.6×10^2	1.4×10^4	4.9×10^5
11	5	1.4×10^1	2.0×10^2	4.6×10^4	2.7×10^6
12	11	9.6	1.9×10^2	1.0×10^5	3.6×10^6
13	3	9.6	3.3×10^2	1.6×10^5	5.9×10^5
14	7	4.3×10^2	2.6×10^2	1.6×10^4	8.0×10^3
15	5	4.7	8.6×10^2	4.9×10^3	5.0×10^6
16	8	4.9×10^1	3.3×10^2	1.4×10^3	9.6×10^3
17[a]	2	5.8×10^2	0	3.9×10^5	5.0×10^5
18[a]	2	4.9×10^1	0	1.1×10^3	9.0×10^3
19	5	contam.	1.2×10^2	contam.	1.6×10^4
20	3	1.9×10^2	0	1.5×10^6	1.7×10^7
21[a]	2	3.6×10^1	4.0×10^2	contam.	0
22[a]	5	8.8×10^1	6.6×10^1	2.6×10^4	2.9×10^4
23	4	0	0	3.6×10^4	7.8×10^6
24[a]	2	1.4×10^2	0	8.8×10^3	3.0×10^5
25	3	1.1×10^4	1.3×10^2	1.8×10^4	2.6×10^2
26[a]	2	1.1×10^3	0	2.9×10^4	1.3×10^5
27	4	1.9×10^1	3.9×10^2	5.2×10^4	3.4×10^6
GM		3.7×10^1	2.5×10^1	1.4×10^4	1.1×10^5
AM		5.8×10^2	1.9×10^2	1.2×10^5	1.9×10^6

[a] Only one biopsy was obtained from these subjects.

though this particular biotype constitutes only 70% of the aerobic coccal types normally cultured from skin of the face [14]. Thus it has been suggested that the aerobic cocci in acne lesions are not random organisms from the normal aerobic flora of the follicles, but that some selectivity in the involvement of one particular biotype may occur [11].

Most studies on the bacteriology of acne lesions have been performed in patients with facial acne. There is a slight possibility that lesions of acne in the back have a different microbial flora. If this were the case, the present analysis of the normal intrafollicular flora on the back could not be related to changes found in acne. However, if lesions of acne on the back contain the same bacteria as in facial acne lesions, then the results of the present study suggest that staphylococci in the acne lesion may be of significance in the pathogenesis of this disease, since staphylococci are not the normal flora in normal follicles.

In recent years, coagulase-negative staphylococci have been discounted as co-agents in the pathogenesis of acne vulgaris. The most important reason for this is probably related to the free fatty acid theory of acne pathogenesis. Several studies have shown that cutaneous staphylococci are not important in the lipolysis of sebaceous triglycerides to free fatty acids [8,15]. However, the question of the importance of the free fatty acids in the pathogenesis of acne remains unresolved. A recent review [16] summarized the reasons why this theory of acne pathogenesis should be reconsidered, and recent studies by Fulton, Weeks, and McCarty [17] further question the validity of this hypothesis.

Even if the free fatty acid theory were modified, the importance of staphylococci in the pathogenesis of acne remains unclear for the reason that 20 to 30% of cocci isolated from pustular acne lesions have been reported to be resistant to tetracycline [18]. Yet tetracycline remains the antibiotic of

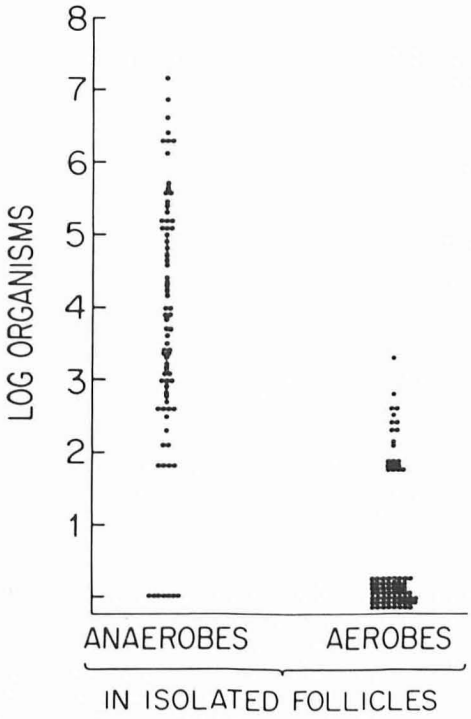

FIG. 3. Range of values of aerobic cocci and anaerobic diphtheroids cultured from 77 isolated follicles from skin of the upper back (of 18 normal subjects).

TABLE V. *Variation of bacterial content of individual isolated follicles in one subject*

Follicle number	Aerobic	Anaerobic
1	0	6.0×10^4
2	0	5.4×10^4
3	0	3.0×10^5
4	0	1.4×10^5
5	6.6×10^1	2.2×10^6
6	1.3×10^2	1.0×10^5
7	0	4.6×10^5
8	0	1.0×10^4
9	0	5.0×10^5
10	0	2.6×10^5
11	0	4.0×10^4

choice in the treatment of pustular acne. The preponderance of evidence favors a role for *P. acnes* in the production of inflammatory lesions in acne, and if tetracycline has its clinical effect in acne through antibacterial action, then this must be related to decreasing the density of *P. acnes* within the follicles. The explanation of the exact role of *P. acnes* in the pathogenesis of acne remains unresolved. Clearly the presence of high numbers of this organism in pilosebaceous follicles by itself does not produce acne in subjects who lack other predisposing factors. It would be of interest to determine whether synergistic activity with *S. epidermidis* is involved in enhancing the inflammatory effect of *P. acnes* in follicles in acne.

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